

IN THE CLAIMS:

1. (Original) A method for producing an infectious, self-replicating, recombinant human parainfluenza virus type 2 (HPIV2) from one or more isolated polynucleotide molecules encoding said HPIV2, comprising:

coexpressing in a cell or cell-free system one or more expression vector(s) comprising a polynucleotide molecule that encodes a partial or complete, polyhexameric recombinant HPIV2 genome or antigenome and one or more polynucleotide molecules encoding PIV N, P and L proteins, thereby producing an infectious HPIV2.

2. (Original) The method of claim 1, wherein the HPIV2 genome or antigenome and the N, P, and L proteins are expressed by multiple expression vectors.

3. (Original) The method of claim 1, wherein at least one of the N, P and L proteins is supplied by coinfection with PIV.

4. (Original) The method of claim 1, wherein the polynucleotide molecule that encodes the recombinant HPIV2 genome or antigenome is cDNA.

5. (Original) The method of claim 1, wherein the infectious HPIV2 particle is a complete virus.

6. (Original) The method of claim 1, wherein one or more of said N, P and L proteins is/are of a heterologous PIV.

7. (Original) The method of claim 1, wherein the polynucleotide molecule encoding the recombinant HPIV2 genome or antigenome encodes the sequence of a wild-type HPIV2 strain.

8. (Original) The method of claim 1, wherein the recombinant HPIV2 genome or antigenome incorporates a recombinantly-introduced restriction site marker or transcriptionally silent point mutation.

9. (Original) The method of claim 1, wherein the polynucleotide molecule encoding the recombinant HPIV2 genome or antigenome incorporates one or more recombinantly-introduced attenuating mutations.

10. (Original) The method of claim 1, wherein the polynucleotide molecule encoding the recombinant HPIV2 genome or antigenome incorporates one or more recombinantly-introduced, temperature sensitive (*ts*) attenuating mutations.

11. (Original) The method of claim 1, wherein the polynucleotide molecule encoding the recombinant HPIV2 genome or antigenome incorporates one or more attenuating mutation(s) identified in a biologically derived mutant PIV strain or other mutant nonsegmented negative stranded RNA virus.
12. (Original) The method of claim 11, wherein the recombinant HPIV2 genome or antigenome incorporates one or more attenuating mutations at one or more amino acid position(s) corresponding to an amino acid position of an attenuating mutation identified in a heterologous, mutant nonsegmented negative stranded RNA virus.
13. (Original) The method of claim 12, wherein the polynucleotide molecule encoding the recombinant HPIV2 genome or antigenome incorporates one or more mutation(s) of HPIV3 JS *cp45*.
14. (Original) The method of claim 13, wherein the recombinant HPIV2 genome or antigenome incorporates one or more attenuating mutation(s) selected from amino acid substitution(s) or deletion(s) at residues 948 and/or 1566 of the HPIV2 L polymerase.
15. (Original) The method of claim 12, wherein the heterologous, mutant nonsegmented negative stranded RNA virus is respiratory syncytial virus (RSV).
16. (Original) The method of claim 15, wherein said attenuating mutation comprises an amino acid substitution or deletion at a corresponding target position Phe460 in the HPIV2 L protein.
17. (Original) The method of claim 12, wherein the heterologous, mutant nonsegmented negative stranded RNA virus is a bovine parainfluenza virus type 3 (BPIV3).
18. (Original) The method of claim 17, wherein said attenuating mutation comprises one or more amino acid substitution(s) or deletion(s) targeting at least Ser1724 in the HPIV2 L protein.
19. (Original) The method of claim 18, wherein said attenuating mutation comprises a two amino acid deletion at Ser1724-1725 in the HPIV2 L protein.
20. (Original) The method of claim 1, wherein the recombinant HPIV2 genome or antigenome incorporates at least one attenuating mutation stabilized by two or three nucleotide changes in a codon specifying the mutation.
21. (Original) The method of claim 1, wherein the recombinant HPIV2 genome or antigenome comprises a nucleotide modification specifying a phenotypic change selected

from a change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

22. (Original) The method of claim 21, wherein the nucleotide modification alters one or more HPIV2 N, P, M, F, HN and/or L genes and/or a 3' leader, 5' trailer, and/or intergenic region within the HPIV2 genome or antigenome.

23. (Original) The method of claim 21, wherein one or more HPIV2 gene(s) is deleted in whole or in part or expression of the gene(s) is reduced or ablated by a mutation in an RNA editing site, by a frameshift mutation, by a mutation that alters a translation start site, by introduction of one or more stop codons in an open reading frame (ORF) of the gene, or by a mutation in a transcription signal.

24. (Original) The method of claim 21, wherein the recombinant HPIV2 genome or antigenome is modified by a partial or complete deletion of an HPIV2 V open reading frame (ORF), or one or more nucleotide change(s) that reduces or ablates expression of said one HPIV2 V ORF.

25. (Original) The method of claim 21, wherein the recombinant HPIV2 genome or antigenome is modified to encode a non-PIV molecule selected from a cytokine, a T-helper epitope, a restriction site marker, or a protein of a microbial pathogen capable of eliciting an immune response in a mammalian host.

26. (Original) The method of claim 25, wherein the recombinant HPIV2 genome or antigenome is modified to encode a cytokine.

27. (Original) The method of claim 1, wherein the recombinant HPIV2 genome or antigenome comprises a partial or complete HPIV2 vector genome or antigenome, wherein said vector genome or antigenome is combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more heterologous pathogen(s) to form a chimeric HPIV2 genome or antigenome.

28. (Original) The method of claim 27, wherein said one or more heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are added as supernumerary gene(s) or genome segment(s) adjacent to or within a noncoding region of the partial or complete HPIV2 vector genome or antigenome, or wherein said one or more heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are substituted for one or more counterpart gene(s) or genome segment(s) in a partial HPIV2 vector genome or antigenome.

29. (Original) The method of claim 27, wherein said one or more heterologous gene(s) or genome segment(s) includes a heterologous regulatory element comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-end signal, editing region, intergenic region, or a 3' or 5' non-coding region.

30. (Original) The method of claim 27, wherein said one or more heterologous pathogens is one or more heterologous PIV(s) and said heterologous gene(s) or genome segment(s) encode(s) one or more PIV N, P, V, M, F, HN and/or L protein(s) or fragment(s) thereof.

31. (Original) The method of claim 27, wherein the heterologous pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, human metapneumovirus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses, human metapneumoviruses, and influenza viruses.

32. (Original) The method of claim 27, wherein the partial or complete HPIV2 vector genome or antigenome is combined with one or more supernumerary heterologous gene(s) or genome segment(s) to form the chimeric HPIV2 genome or antigenome.

33. (Original) The method of claim 32, wherein said one or more supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV1 HN, HPIV2 F, HPIV3 HN, HPIV3 F, and measles HA.

34. (Original) The method of claim 27, wherein the HPIV2 vector genome or antigenome incorporates one or more gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous HPIV and one or more gene(s) or genome segment(s) encoding one or more heterologous antigenic determinant(s) of a non-PIV pathogen selected from measles virus, respiratory syncytial virus, mumps virus, human papilloma virus, type 1 or type 2 human immunodeficiency virus, herpes simplex virus, cytomegalovirus, rabies virus, human metapneumovirus, Epstein Barr Virus, filovirus, bunyavirus, flavivirus, alphavirus, human metapneumoviruses, and influenza virus.

35. (Original) The method of claim 27, wherein the heterologous gene or genome segment is added or substituted at a position corresponding to a wild-type gene order position of a counterpart gene or genome segment within the partial or complete HPIV2 vector genome or antigenome.

36. (Original) The method of claim 27, wherein the heterologous gene or genome segment is added or substituted at a position that is more promoter-proximal or promoter-distal compared to a wild-type gene order position of a counterpart gene or genome segment within the partial or complete HPIV2 vector genome or antigenome.

37. (Original) The method of claim 27, wherein the HPIV2 vector genome or antigenome is modified to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments, or epitopes of a heterologous PIV or non-PIV pathogen to form the chimeric genome or antigenome.

38. (Original) The method of claim 37, wherein the HPIV2 vector genome or antigenome is modified to encode a chimeric glycoprotein incorporating one or more antigenic domains, fragments, or epitopes from a second, antigenically distinct PIV to form the chimeric genome or antigenome.

39. (Original) The method of claim 37, wherein the chimeric genome or antigenome encodes a chimeric virus or chimeric glycoprotein having antigenic domains, fragments, or epitopes from two or more HPIVs.

40. (Original) The method of claim 37, wherein the heterologous genome segment encodes a glycoprotein cytoplasmic, transmembrane or ectodomain which is substituted for a corresponding glycoprotein domain in the HPIV2 vector genome or antigenome.

41. (Original) The method of claim 37, wherein one or more heterologous genome segment(s) of a second, antigenically distinct HPIV encoding said one or more antigenic domains, fragments, or epitopes is/are substituted within a HPIV2 vector genome or antigenome to encode said chimeric glycoprotein.

42. (Original) The method of claim 37, wherein said one or more heterologous genome segment(s) are selected from ectodomains of HPIV1 and/or HPIV3 HN and/or F glycoproteins.

43. (Original) The method of claim 27, wherein the chimeric HPIV2 genome or antigenome is modified by introduction of one or more attenuating mutations identified in a biologically derived mutant PIV or other mutant nonsegmented negative stranded RNA virus.

44. (Original) The method of claim 43, wherein the polynucleotide molecule encoding the recombinant HPIV2 genome or antigenome incorporates one or more mutation(s) of HPIV3 JS *cp45*.

45. (Original) The method of claim 44, wherein the recombinant HPIV2 genome or antigenome incorporates one or more attenuating mutation(s) selected from amino acid substitution(s) or deletion(s) at residues 948 and/or 1566 of the HPIV2 L polymerase.

46. (Original) The method of claim 43, wherein the heterologous, mutant nonsegmented negative stranded RNA virus is respiratory syncytial virus (RSV).

47. (Original) The method of claim 46, wherein said attenuating mutation comprises an amino acid substitution or deletion at a corresponding target position Phe460 in the HPIV2 L protein.

48. (Original) The method of claim 43, wherein the heterologous, mutant nonsegmented negative stranded RNA virus is a bovine parainfluenza virus type 3 (BPIV3).

49. (Original) The method of claim 48, wherein said attenuating mutation comprises one or more amino acid substitution(s) or deletion(s) targeting at least Ser1724 in the HPIV2 L protein.

50. (Original) The method of claim 49, wherein said attenuating mutation comprises a two amino acid deletion at Ser1724-1725 in the HPIV2 L protein.

51. (Original) The method of claim 43, wherein the chimeric HPIV2 genome or antigenome incorporates at least one attenuating mutation stabilized by two or three nucleotide changes in a codon specifying the mutation.

52. (Original) The method of claim 43, wherein said one or combination of mutation(s) in the chimeric HPIV2 genome or antigenome is/are located in the partial or complete HPIV2 vector genome or antigenome.

53. (Original) The method of claim 41, wherein said one or more attenuating mutations is/are located in the heterologous gene(s) or genome segment(s) incorporated in the chimeric genome or antigenome.

54. (Original) The method of claim 27, wherein the chimeric HPIV2 genome or antigenome is further modified to incorporate an additional nucleotide modification specifying a phenotypic change selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

55. (Original) The method of claim 54, wherein the chimeric HPIV2 genome or antigenome is further modified such that one or more HPIV2 gene(s) is/are deleted in whole or in part or expression of the gene(s) is reduced or ablated by a mutation in an RNA editing

site, by a frameshift mutation, by a mutation that alters a translation start site, by introduction of one or more stop codons in an open reading frame (ORF) of the gene, or by a mutation in a transcription signal.

56. (Original) The method of claim 55, wherein the chimeric HPIV2 genome or antigenome is modified by a partial or complete deletion of an HPIV2 V open reading frame (ORF) or by one or more nucleotide change(s) that reduces or ablates expression of said HPIV2 V ORF.

57. (Original) The method of claim 54, wherein the chimeric HPIV2 genome or antigenome is further modified to encode a non-PIV molecule selected from a cytokine, a T-helper epitope, a restriction site marker, or a protein of a microbial pathogen capable of eliciting an immune response in a mammalian host.

58. (Original) The method of claim 27, wherein the chimeric HPIV2 genome or antigenome comprises a partial or complete HPIV2 vector genome or antigenome combined with one or more heterologous genes or genome segments from a bovine parainfluenza virus type 3 virus (BPIV3) to form a human-bovine chimeric HPIV2 genome or antigenome.

59. (Original) The method of claim 58, wherein the partial or complete HPIV2 vector genome or antigenome is combined with one or more heterologous gene(s) or genome segment(s) of a N, P, L, or M gene of a BPIV3 to form a human-bovine chimeric genome or antigenome.

60. (Original) The method of claim 58, wherein a bovine PIV (BPIV) N, M, L, or P open reading frame (ORF) or a genome segment thereof is substituted for a counterpart HPIV2 N, M, L, or P ORF or genome segment to form a chimeric HPIV2-BPIV3 genome or antigenome.

61. (Original) The method of claim 27, wherein the chimeric HPIV2 genome or antigenome is modified by addition or substitution of one or more additional heterologous gene(s) or genome segment(s) from a bovine parainfluenza virus type 3 virus (BPIV3) within the partial or complete HPIV2 vector genome or antigenome to increase genetic stability or alter attenuation, reactogenicity or growth in culture of the recombinant virus.

62. (Original) The method of claim 27, wherein the chimeric HPIV2 genome or antigenome incorporates a polynucleotide insertion of between 150 nucleotides (nts) and 4,000 nucleotides in length in a non-coding region (NCR) of the genome or antigenome or as a separate gene unit (GU), said polynucleotide insertion lacking a complete open reading frame (ORF) and specifying an attenuated phenotype in said recombinant HPIV2.

63. (Original) The method of claim 62, wherein said polynucleotide insert is introduced into the HPIV2 genome or antigenome in a reverse, non-sense orientation whereby the insert does not encode protein.
64. (Original) The method of claim 62, wherein said recombinant HPIV2 replicates efficiently *in vitro* and exhibits an attenuated phenotype *in vivo*.
65. (Original) The method of claim 62, wherein said polynucleotide insertion adds a total length of foreign sequence to the recombinant HPIV2 genome or antigenome of 30% to 50% or greater compared to a wild-type HPIV2 genome length.
66. (Original) The method of claim 62, wherein said polynucleotide insertion specifies an attenuation phenotype of the recombinant HPIV2 which exhibits at least a 10-to 100-fold decrease in replication in the upper and/or lower respiratory tract.
67. (Original) An isolated, infectious, self-replicating, recombinant human parainfluenza virus type 2 (HPIV2) comprising a PIV major nucleocapsid (N) protein, a PIV nucleocapsid phosphoprotein (P), a PIV large polymerase protein (L), and a partial or complete, polyhexameric recombinant HPIV2 genome or antigenome.
68. (Original) The recombinant HPIV2 of claim 67, wherein at least one of the N, P and L proteins is of a different HPIV or a bovine parainfluenza virus type 3 virus (BPIV3).
69. (Original) The recombinant HPIV2 of claim 67, wherein one or more of said N, P and L proteins is/are of HPIV3.
70. (Original) The recombinant HPIV2 of claim 67, wherein the recombinant HPIV2 genome or antigenome is encoded by cDNA.
71. (Original) The recombinant HPIV2 of claim 67, wherein the infectious HPIV2 particle is a complete virus.
72. (Original) The recombinant HPIV2 of claim 67, wherein the recombinant HPIV2 genome or antigenome is of a wild-type HPIV2 strain.
73. (Original) The recombinant HPIV2 of claim 67, wherein the recombinant HPIV2 genome or antigenome incorporates one or more recombinantly-introduced attenuating mutations.
74. (Original) The recombinant HPIV2 of claim 67, wherein the recombinant HPIV2 genome or antigenome incorporates one or more recombinantly-introduced attenuating mutations at one or more amino acid position(s) corresponding to an amino acid

position of an attenuating mutation identified in a heterologous, mutant nonsegmented negative stranded RNA virus.

75. (Original) The recombinant HPIV2 of claim 74, wherein the polynucleotide molecule encoding the recombinant HPIV2 genome or antigenome incorporates one or more mutation(s) of HPIV3 JS *cp45*.

76. (Original) The recombinant HPIV2 of claim 75, wherein the recombinant HPIV2 genome or antigenome incorporates one or more attenuating mutation(s) selected from amino acid substitution(s) or deletion(s) at residues 948 and/or 1566 of the HPIV2 L polymerase.

77. (Original) The recombinant HPIV2 of claim 74, wherein the heterologous, mutant nonsegmented negative stranded RNA virus is respiratory syncytial virus (RSV).

78. (Original) The recombinant HPIV2 of claim 77, wherein said attenuating mutation comprises an amino acid substitution or deletion at a corresponding target position Phe460 in the HPIV2 L protein.

79. (Original) The recombinant HPIV2 of claim 74, wherein the heterologous, mutant nonsegmented negative stranded RNA virus is a bovine parainfluenza virus type 3 (BPIV3).

80. (Original) The recombinant HPIV2 of claim 79, wherein said attenuating mutation comprises one or more amino acid substitution(s) or deletion(s) targeting at least Ser1724 in the HPIV2 L protein.

81. (Original) The recombinant HPIV2 of claim 80, wherein said attenuating mutation comprises a two amino acid deletion at Ser1724-1725 in the HPIV2 L protein.

82. (Original) The recombinant HPIV2 of claim 74, wherein the recombinant HPIV2 genome or antigenome incorporates at least one attenuating mutation stabilized by two or three nucleotide changes in a codon specifying the mutation.

83. (Original) The recombinant HPIV2 of claim 67, wherein the recombinant HPIV2 genome or antigenome comprises a nucleotide modification specifying a phenotypic change selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

84. (Original) The recombinant HPIV2 of claim 83, wherein the additional nucleotide modification alters one or more HPIV2 N, P, M, F, HN and/or L genes and/or a 3' leader, 5' trailer, and/or intergenic region within the HPIV2 genome or antigenome.

85. (Original) The recombinant HPIV2 of claim 83, wherein one or more HPIV2 gene(s) is deleted in whole or in part or expression of the gene(s) is reduced or ablated by a mutation in an RNA editing site, by a frameshift mutation, by a mutation that alters a translation start site, by introduction of one or more stop codons in an open reading frame (ORF) of the gene, or by a mutation in a transcription signal.

86. (Original) The recombinant HPIV2 of claim 85, wherein the recombinant HPIV2 genome or antigenome is modified by a partial or complete deletion of an HPIV2 V open reading frame (ORF), or one or more nucleotide change(s) that reduces or ablates expression of said one HPIV2 V ORF.

87. (Original) The recombinant HPIV2 of claim 83, wherein the recombinant HPIV2 genome or antigenome is modified to encode a non-PIV molecule selected from a cytokine, a T-helper epitope, a restriction site marker, or a protein of a microbial pathogen capable of eliciting an immune response in a mammalian host.

88. (Original) The recombinant HPIV2 of claim 87, wherein the recombinant HPIV2 genome or antigenome is modified to encode a cytokine.

89. (Original) The recombinant HPIV2 of claim 67, wherein the recombinant HPIV2 genome or antigenome comprises a partial or complete HPIV2 vector genome or antigenome, wherein said vector genome or antigenome is combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more heterologous pathogen(s) to form a chimeric HPIV2 genome or antigenome.

90. (Original) The recombinant HPIV2 of claim 89, wherein said one or more heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are added as supernumerary gene(s) or genome segment(s) adjacent to or within a noncoding region of the partial or complete HPIV2 vector genome or antigenome, or wherein said one or more heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are substituted for one or more counterpart gene(s) or genome segment(s) in a partial HPIV2 vector genome or antigenome.

91. (Original) The recombinant HPIV2 of claim 89, wherein said one or more heterologous gene(s) or genome segment(s) includes a heterologous regulatory element comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-end signal, editing region, intergenic region, or a 3' or 5' non-coding region.

92. (Original) The recombinant HPIV2 of claim 89, wherein said one or more heterologous pathogens is/are one or more heterologous PIV(s) and said heterologous gene(s)

or genome segment(s) encode(s) one or more PIV N, P, V, M, F, HN and/or L protein(s) or fragment(s) thereof.

93. (Original) The recombinant HPIV2 of claim 89, wherein the heterologous pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, human metapneumovirus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses, human metapneumoviruses, and influenza viruses.

94. (Original) The recombinant HPIV2 of claim 89, wherein the partial or complete HPIV2 vector genome or antigenome is combined with one or more supernumerary heterologous gene(s) or genome segment(s) to form the chimeric HPIV2 genome or antigenome.

95. (Original) The recombinant HPIV2 of claim 94, wherein said one or more supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV1 HN, HPIV2 F, HPIV3 HN, HPIV3 F, and measles HA.

96. (Original) The recombinant HPIV2 of claim 89, wherein the HPIV2 vector genome or antigenome incorporates one or more gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous HPIV and one or more gene(s) or genome segment(s) encoding one or more heterologous antigenic determinant(s) of a non-PIV pathogen selected from measles virus, respiratory syncytial virus (RSV), mumps virus, human papilloma virus, type 1 or type 2 human immunodeficiency virus, herpes simplex virus, cytomegalovirus, rabies virus, human metapneumovirus, Epstein Barr Virus, filovirus, bunyavirus, flavivirus, alphavirus, human metapneumoviruses, and influenza virus.

97. (Original) The recombinant HPIV2 of claim 96, wherein the heterologous pathogen is RSV and the heterologous antigenic determinant(s) is/are selected from the RSV G and F proteins and antigenic domains, fragments and epitopes thereof.

98. (Original) The recombinant HPIV2 of claim 89, wherein the heterologous gene or genome segment is added or substituted at a position corresponding to a wild-type gene order position of a counterpart gene or genome segment within the partial or complete HPIV2 vector genome or antigenome.

99. (Original) The recombinant HPIV2 of claim 89, wherein the heterologous gene or genome segment is added or substituted at a position that is more promoter-proximal

or promoter-distal compared to a wild-type gene order position of a counterpart gene or genome segment within the partial or complete HPIV2 vector genome or antigenome.

100. (Original) The recombinant HPIV2 of claim 89, wherein the HPIV2 vector genome or antigenome is modified to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments, or epitopes of a heterologous PIV or non-PIV pathogen to form the chimeric genome or antigenome.

101. (Original) The recombinant HPIV2 of claim 100, wherein the HPIV2 vector genome or antigenome is modified to encode a chimeric glycoprotein incorporating one or more antigenic domains, fragments, or epitopes from a second, antigenically distinct PIV to form the chimeric genome or antigenome.

102. (Original) The recombinant HPIV2 of claim 101, wherein the chimeric genome or antigenome encodes a chimeric virus or chimeric glycoprotein having antigenic domains, fragments, or epitopes from two or more HPIVs.

103. (Original) The recombinant HPIV2 of claim 100, wherein the heterologous genome segment encodes a glycoprotein cytoplasmic, transmembrane or ectodomain which is substituted for a corresponding glycoprotein domain in the HPIV2 vector genome or antigenome.

104. (Original) The recombinant HPIV2 of claim 100, wherein said one or more heterologous genome segment(s) are selected from ectodomains of HPIV1 and/or HPIV3 HN and/or F glycoproteins.

105. (Original) The recombinant HPIV2 of claim 89, wherein the chimeric HPIV2 genome or antigenome incorporates one or more recombinantly-introduced attenuating mutations at one or more amino acid position(s) corresponding to an amino acid position of an attenuating mutation identified in a heterologous, mutant nonsegmented negative stranded RNA virus.

106. (Original) The recombinant HPIV2 of claim 105, wherein the polynucleotide molecule encoding the chimeric HPIV2 genome or antigenome incorporates one or more mutation(s) of HPIV3 JS *cp45*.

107. (Original) The recombinant HPIV2 of claim 106, wherein the chimeric HPIV2 genome or antigenome incorporates one or more attenuating mutation(s) selected from amino acid substitution(s) or deletion(s) at residues 948 and/or 1566 of the HPIV2 L polymerase.

108. (Original) The recombinant HPIV2 of claim 105, wherein the heterologous, mutant nonsegmented negative stranded RNA virus is respiratory syncytial virus (RSV).

109. (Original) The recombinant HPIV2 of claim 108, wherein said attenuating mutation comprises an amino acid substitution or deletion at a corresponding target position Phe460 in the HPIV2 L protein.

110. (Original) The recombinant HPIV2 of claim 105, wherein the heterologous, mutant nonsegmented negative stranded RNA virus is a bovine parainfluenza virus type 3 (BPIV3).

111. (Original) The recombinant HPIV2 of claim 110, wherein said attenuating mutation comprises one or more amino acid substitution(s) or deletion(s) targeting at least Ser1724 in the HPIV2 L protein.

112. (Original) The recombinant HPIV2 of claim 111, wherein said attenuating mutation comprises a two amino acid deletion at Ser1724-1725 in the HPIV2 L protein.

113. (Original) The recombinant HPIV2 of claim 105, wherein the recombinant HPIV2 genome or antigenome incorporates at least one attenuating mutation stabilized by two or three nucleotide changes in a codon specifying the mutation.

114. (Original) The recombinant HPIV2 of claim 105, wherein said one or more mutation(s) in the chimeric HPIV2 genome or antigenome is/are located in the partial or complete HPIV2 vector genome or antigenome.

115. (Original) The recombinant HPIV2 of claim 114, wherein said one or more mutation(s) in the chimeric HPIV1 genome or antigenome is/are located in the heterologous gene(s) or genome segment(s) incorporated in the chimeric genome or antigenome.

116. (Original) The recombinant HPIV2 of claim 89, wherein the chimeric HPIV2 genome or antigenome is modified to incorporate an additional nucleotide modification specifying a phenotypic change selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

117. (Original) The recombinant HPIV2 of claim 116, wherein the chimeric HPIV2 genome or antigenome is modified such that one or more HPIV2 gene(s) is/are deleted in whole or in part or expression of the gene(s) is reduced or ablated by a mutation in an RNA editing site, by a frameshift mutation, by a mutation that alters a translation start site,

by introduction of one or more stop codons in an open reading frame (ORF) of the gene, or by a mutation in a transcription signal.

118. (Original) The recombinant HPIV2 of claim 117, wherein the chimeric HPIV2 genome or antigenome is modified by a partial or complete deletion of an HPIV2 V open reading frame (ORF) or by one or more nucleotide change(s) that reduces or ablates expression of said HPIV2 V ORF.

119. (Original) The recombinant HPIV2 of claim 116, wherein the chimeric HPIV2 genome or antigenome is further modified to encode a non-PIV molecule selected from a cytokine, a T-helper epitope, a restriction site marker, or a protein of a microbial pathogen capable of eliciting an immune response in a mammalian host.

120. (Original) The recombinant HPIV2 of claim 89, wherein the chimeric HPIV2 genome or antigenome comprises a partial or complete HPIV2 vector genome or antigenome combined with one or more heterologous genes or genome segments from a bovine parainfluenza virus type 3 virus (BPIV3) to form a human-bovine chimeric HPIV2 genome or antigenome.

121. (Original) The recombinant HPIV2 of claim 120, wherein the partial or complete HPIV2 vector genome or antigenome is combined with one or more heterologous gene(s) or genome segment(s) of a N, P, L, or M gene of a BPIV3 to form a human-bovine chimeric genome or antigenome.

122. (Original) The recombinant HPIV2 of claim 120, wherein a BPIV3 N, M, L, or P open reading frame (ORF) or a genome segment thereof is substituted for a counterpart HPIV2 N, M, L, or P ORF or genome segment to form a chimeric HPIV2-BPIV3 genome or antigenome.

123. (Original) The recombinant HPIV2 of claim 89, wherein the chimeric HPIV2 genome or antigenome is further modified by addition or substitution of one or more additional heterologous gene(s) or genome segment(s) from a bovine parainfluenza virus type 3 virus (BPIV3) within the partial or complete HPIV2 vector genome or antigenome to increase genetic stability or alter attenuation, reactogenicity or growth in culture of the recombinant virus.

124. (Original) The recombinant HPIV2 of claim 67, wherein the recombinant HPIV2 genome or antigenome incorporates a polynucleotide insertion of between 150 nucleotides (nts) and 4,000 nucleotides in length in a non-coding region (NCR) of the genome or antigenome or as a separate gene unit (GU), said polynucleotide insertion lacking

a complete open reading frame (ORF) and specifying an attenuated phenotype in said recombinant HPIV2.

125. (Original) The recombinant HPIV2 of claim 124, wherein said polynucleotide insert is introduced into the HPIV2 genome or antigenome in a reverse, non-sense orientation whereby the insert does not encode protein.

126. (Original) The recombinant HPIV2 of claim 124, wherein said recombinant HPIV2 replicates efficiently *in vitro* and exhibits an attenuated phenotype *in vivo*.

127. (Original) The recombinant HPIV2 of claim 124, wherein said polynucleotide insertion adds a total length of foreign sequence to the recombinant HPIV2 genome or antigenome of 30% to 50% or greater compared to a wild-type HPIV2 genome length.

128. (Original) The recombinant HPIV2 of claim 124, wherein said polynucleotide insertion specifies an attenuation phenotype of the recombinant HPIV2 which exhibits at least a 10-to 100-fold decrease in replication in the upper and/or lower respiratory tract. wherein one or more of the PIV N, P, and/or L proteins are of a heterologous PIV.

129. (Original) An immunogenic composition comprising an immunogenically effective amount of an isolated, infectious, self-replicating, recombinant human parainfluenza virus type 2 (HPIV2) in a pharmaceutically acceptable carrier, said HPIV2 comprising a polyhexameric, recombinant HPIV2 genome or antigenome, a PIV N protein, a PIV P protein, and a PIV L protein.

130-182. Cancelled.

183. (Original) A method for stimulating the immune system of a mammalian subject to induce an immune response in the subject against PIV which comprises administering to the subject an immunologically sufficient amount of an isolated, infectious, self-replicating, recombinant human parainfluenza virus type 2 (HPIV2) comprising a PIV major nucleocapsid (N) protein, a PIV nucleocapsid phosphoprotein (P), a PIV large polymerase protein (L), and a partial or complete, polyhexameric recombinant HPIV2 genome or antigenome.

184-231. Cancelled

232. (Original) A method for sequential immunization to stimulate the immune system of a mammalian subject to induce an immune response against multiple pathogens comprising administering to the subject an immunologically sufficient amount of a first HPIV and subsequently administering to the subject an immunologically sufficient amount of a

second HPIV, wherein at least one of said first and second HPIVs comprises a partial or complete HPIV2 vector genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more heterologous pathogen(s) to form a chimeric HPIV1 genome or antigenome.

233-254. Cancelled.

255. (Original) An isolated polynucleotide comprising a partial or complete, polyhexameric recombinant human parainfluenza virus type 2 (HPIV2) genome or antigenome modified by one or more attenuating mutations that are recombinantly introduced into said HPIV2 genome or antigenome.

256-277. Cancelled.

278. (Original) An expression vector comprising an operably linked transcriptional promoter, a polynucleotide sequence comprising a partial or complete, polyhexameric recombinant human parainfluenza virus type 2 (HPIV2) genome or antigenome modified by one or more attenuating mutations that are recombinantly introduced into said HPIV2 genome or antigenome, and a transcriptional terminator.

279-294. Cancelled.